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RESEARCH ARTICLE

Genetic resiliency and the Black Death: No apparent loss of mitogenomic diversity due to the Black Death in medieval London and Denmark

Jennifer Klunk[1,2] | Ana T. Duggan[1,3] | Rebecca Redfern[4] | Julia Gamble[5] | Jesper L. Boldsen[6] | G. Brian Golding[2] | Brittany S. Walter[7] | Katherine Eaton[1,3] | Julianna Stangroom[1,3] | Jean-Marie Rouillard[8,9] | Alison Devault[8] | Sharon N. DeWitte[10] | Hendrik N. Poinar[1,2,3,11,12]

[1]McMaster Ancient DNA Centre, McMaster University, Hamilton, Ontario, Canada

[2]Department of Biology, McMaster University, Hamilton, Ontario, Canada

[3]Department of Anthropology, McMaster University, Hamilton, Ontario, Canada

[4]Center for Human Bioarchaeology, Museum of London, London, UK

[5]Department of Anthropology, University of Manitoba, Winnipeg, Manitoba

[6]Department of Forensic Medicine, Unit of Anthropology (ADBOU), University of Southern Denmark, Odense, Denmark

[7]Defense POW/MIA Accounting Agency Laboratory, Offutt AFB, Omaha, Nebraska

[8]Arbor Biosciences, Ann Arbor, Michigan

[9]Department of Chemical Engineering, University of Michigan Ann Arbor, Ann Arbor, Michigan

[10]Department of Anthropology, University of South Carolina, Columbia, South Carolina

[11]Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada

[12]Michael G. DeGroote Institute of Infectious Disease Research, McMaster University, Hamilton, Ontario, Canada

Correspondence
Jennifer Klunk, McMaster Ancient DNA Center, Chester New Hall 412, 1280 Main Street West, Hamilton, Ontario, Canada, L8S4L8,
Email: klunkjm@mcmaster.ca and Hendrik N. Poniar, McMaster Ancient DNA Center, Chester New Hall 537, 1280 Main Street West, Hamilton, Ontario, Canada, L8S4L8,
Email: poinarh@mcmaster.ca

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Abstract

Objectives: In the 14th century AD, medieval Europe was severely affected by the Great European Famine as well as repeated bouts of disease, including the Black Death, causing major demographic shifts. This high volatility led to increased mobility and migration due to new labor and economic opportunities, as evidenced by documentary and stable isotope data. This study uses ancient DNA (aDNA) isolated from skeletal remains to examine whether evidence for large-scale population movement can be gleaned from the complete mitochondrial genomes of 264 medieval individuals from England (London) and Denmark.

Materials and Methods: Using a novel library-conserving approach to targeted capture, we recovered 264 full mitochondrial genomes from the petrous portion of the temporal bones and teeth and compared genetic diversity across the medieval period within and between English (London) and Danish populations and with contemporary populations through population pairwise Φ_{ST} analysis.

Results: We find no evidence of significant differences in genetic diversity spatially or temporally in our dataset, yet there is a high degree of haplotype diversity in our medieval samples with little exact sequence sharing.

Discussion: The mitochondrial genomes of both medieval Londoners and medieval Danes suggest high mitochondrial diversity before, during and after the Black Death. While our mitochondrial genomic data lack geographically correlated signals, these data could be the result of high, continual female migration before and after the Black Death or may simply indicate a large

female effective population size unaffected by the upheaval of the medieval period. Either scenario suggests a genetic resiliency in areas of northwestern medieval Europe.

KEYWORDS

ancient DNA, medieval London and Denmark, migration, mitochondrial DNA

1 | INTRODUCTION

1.1 | Disaster and disruption in 14th century Europe

During the 14th century AD, Europe experienced a series of catastrophic events that produced severe spikes in mortality and caused major social and economic upheaval. By comparison, between the 11th and 13th centuries AD, Europe experienced stable population growth, in part due to warmer temperatures, which increased total arable land, and thereby total food availability (Campbell, 2016; Fagan, 2008). In England alone, the population is estimated to have increased from approximately 1.7 million in 1086 AD to approximately 4.75 million by 1290 AD (Campbell, 2016). The population of Denmark was smaller in comparison and is estimated to have been approximately 1.3 million in 1200 AD and to have increased to approximately 2 million at the beginning of the 13th century AD (Hybel & Poulsen, 2007). However, beginning in the late 13th century AD, increasing climate instability and epidemics led to repeated crop failures, livestock deaths, and ultimately famine throughout the early years of the 14th century AD, most notably the Great European Famine (Campbell, 2016; Jordan, 1997). Between 1347 and 1352 AD, on the heels of this environmental and food collapse, came the Black Death, an epidemic estimated to have killed between 30 and 50% of Europe's population (Benedictow, 2004). This mortality rate drastically reduced the number of workers available in urban (and rural) areas leading to occupational vacancies. In response, people, especially young women, were drawn in from rural settings or from even further abroad to city centers in order to capitalize on opportunities to find work and/or earn higher wages (Goldberg, 2004; Kowaleski, 2014; Redfern & Hefter, 2019). The effects of this tumultuous period on genetic diversity remain uncharacterized. This study investigates what, if any, effects of the Black Death can be observed in the mitochondrial genomic diversity of populations before, during and after the pandemic struck London, England (1348) and Denmark (shortly thereafter in late 1349) with respect to population mortality and rural–urban migration (Lenz & Hybel, 2016).

1.2 | The medieval city of London

To study the effects of mortality and migration on an urban population, we chose the city of London, which provides one of the best case studies to examine the effects of the Black Death. Specifically, the emergency mass burial ground at East Smithfield (MIN86 period 1) has proven extremely valuable for studying Black Death mortality, due to its tight time frame and records of its use (DeWitte & Hughes-Morey, 2012; DeWitte & Wood, 2008; Gowland & Chamberlain, 2005; Grainger, Hawkins, Cowal, & Mikulski, 2008; Margerison & Knüsel, 2002; Waldron, 2001). The burial ground was established in late-1348 AD as a response to the mortality of the Black Death and was used until 1350 AD (Grainger et al., 2008). When the pandemic receded, the land was given to the Cistercian abbey of St. Mary Graces (MIN86 period 2; Grainger & Phillpotts, 2011). Genetic evidence of the bacterium that causes modern-day plague, *Yersinia pestis*, has been isolated from several individuals interred in East Smithfield, suggesting this pathogen was a contributing, but not necessarily exclusive, causative factor in the Black Death (Bos et al., 2011; Schuenemann et al., 2011). Other church or parish cemeteries in London provide comparative data points from both before and after the plague, including the parish cemetery of St. Nicholas Shambles (GPO75, 11th–12th centuries AD) and the Augustinian hospital and priory of St. Mary Spital (SRP98, 12th–16th centuries AD) (Connell, Jones, Redfern, & Walker, 2012; White & Dyson, 1988).

Migration into urban centers of Britain, including London, has been studied with non-DNA-based techniques. Stable isotope studies of remains from several cemeteries in London, including some of the cemeteries used here, show evidence of migrants from areas outside the urban center of London (Kendall, Montgomery, Evans, Stantis, & Mueller, 2013; Lakin, 2010; Walter, 2017). Strontium and oxygen isotope analysis on a sample set of 30 individuals buried in East Smithfield identified five as potential migrants, likely from other locales within Britain (Kendall et al., 2013). A recent study forensic analysis attempting to determine the ancestry of a subset of individuals from this cemetery found the presence of several individuals with presumed Black African ancestry buried at East Smithfield, suggesting that this population may have been diverse (Redfern & Hefter, 2019). Other studies have looked for changes and differences in diet over time and between groups using carbon and nitrogen isotopes to identify potential migrants. Walter (2017) integrated carbon and nitrogen isotopic results from bone and incremental dentine analyses to identify potential migrants at St. Mary Spital. Abrupt changes and differences between early and late-life isotope values suggest a sudden change in diet likely synchronous with a migration event for several individuals in late-medieval London. Lakin (2010) also used carbon and nitrogen isotopes to study diet at St. Mary Spital and St. Nicholas Shambles and found that young females had a statistically significant difference in diet compared to older females. One possible interpretation of these data is that young women were frequently migrating to urban areas to seek work in the medieval periods and thus consuming different foods. Documentary sources such as wills, apprenticeship records, and subsidiary rolls show that London drew migrants from a

wide catchment area, most likely due to the availability of work and apprenticeships in the city (Goldberg, 2004). A paleopathological study of children and adolescents from rural and urban contexts in Britain suggests that young people, especially women, were relocating to urban areas for work opportunities after the Black Death (Lewis, 2016). Walter and DeWitte (2017) analyze trends in survivorship and risk of mortality in individuals from a rural and an urban cemetery in late medieval England. They found no significant difference in the risk of mortality for rural versus urban males, but relative to rural counterparts, the risk of mortality for urban females was greater, which suggests that more females were likely migrating to urban environments and experiencing the higher risks associated with urban living such as novel infectious disease, poverty, and famine. In the present study, we sought to determine whether these migration events were detectable by comparing the changes in mitogenomic diversity of medieval Londoners and Danes who died before, during, or after the first wave of the second pandemic.

1.3 | Denmark in the medieval period

In order to characterize the effects of the Black Death on both urban and nonurban settings, we sampled from several medieval cemeteries across Denmark, which span the Jutland peninsula as well as several islands and were primarily in use from the early 12th century AD to the mid-16th century AD. Both urban and rural locales were sampled in order to compare the two settings within a single geographical context. The hypervariable regions of mitochondrial DNA (mtDNA) from some of the medieval Danes included in this study have been previously sequenced (Krause-Kyora et al., 2018). The present study expands on this dataset by providing full mtDNA genomes for that subset of overlapping individuals (n = 10), increasing the resolution to which the haplotypes can be called.

The urban cemeteries included in this study were located in the towns of Ribe, Horsens, and Viborg. While these were small cities in comparison to London, as the population density of Denmark was much lower, they were important centers of commerce and administration for Denmark (Hybel & Poulsen, 2007). For example, Ribe was located at a port and was one of the earliest cities founded in Denmark, due to its importance in trade, and later became the seat of a bishopric, as did Viborg, which was also a seat of government assemblies (Sawyer & Sawyer, 1993). There are few records associated with the use of the rural cemeteries, even though the majority of the Danish population in the medieval period lived in rural areas. The population residing in urban areas probably did not exceed 10% before the end of the Middle Ages (~1536 AD; Hybel & Poulsen, 2007). However, many of these cemeteries have been studied extensively from a bioarchaeological perspective, and these studies have provided insight into topics like disease burden and stress (Boldsen, 2005; Gamble, Boldsen, & Hoppa, 2017).

Like London, stable isotope evidence has been used to study both mobility and dietary changes throughout the medieval period in Denmark. Yoder (2010) examined carbon and nitrogen values from remains spanning the medieval period from three cemeteries in Jutland and found little evidence of dietary change through time, but some evidence of regional differences in diet. Gough (2014) and Duignan (2015) measured variation in oxygen and strontium isotopes, respectively, at the cemeteries of Sejet and Ole Wormsgade (rural and urban cemeteries associated with Horsens), and found evidence of only four potential migrants out of a total of 55 individuals studied, three of which were female. By comparing Danish urban and rural populations, we can examine whether there were differences in genetic diversity of these contexts within a larger geographic area.

We recognize that although we are comparing populations of London and Denmark, differences in conditions and behavior between rural and urban communities may not have been as extreme in Denmark as those between London and its rural surroundings due to differences in population sizes. Prior to the Black Death (1348 AD), the population of London is estimated to have been between 45,000 and 80,000, whereas estimates for large cities in Denmark are in the low thousands, (e.g., the upper estimate for Ribe is 4,000 inhabitants by the middle of the 13th century AD; Grainger et al., 2008; Jacobsen, 1986) Despite the smaller urban population sizes, differences between Danish rural and urban communities existed. For example, Boldsen (1997) found that individuals from urban centers were on average taller than their rural counterparts and attributed this difference to increased migration into cities and insularity in rural areas. Following up on this work, Boldsen and Søgaard (1998) refined this result to the mobile population residing on the outskirts of the city as the taller population, not those living in the city center. We provide the first mitochondrial genomic data spanning this important and complex medieval time frame from both Danish and English (from London) individuals.

2 | MATERIALS AND METHODS

2.1 | Sample selection and categorization

A complete description of the methods summarized below can be found in the Supporting Information. Based on performance in a quantitative PCR (qPCR) screen for single-copy nuclear DNA and a subsequent nuclear DNA capture experiment, 145 individuals from three cemeteries in London and 134 individuals from six localities across Denmark were selected for mitochondrial DNA capture. DNA was extracted from teeth (n = 113) and petrous portions of the temporal bone (n = 32) in the London material and from dental pulp in the Danish material (Figure 1, Table 1, Supporting Information Table S1). The samples from London were dated to before, during, and after the Black Death based on documented cemetery use, stratigraphy, and radiocarbon dating depending on the cemetery (Connell et al., 2012; Grainger et al., 2008; Grainger & Phillpotts, 2011; White & Dyson, 1988).

The Danish samples were dated on the basis of arm position, a relative dating technique that is commonly used for the medieval period in Denmark (see Supporting Information Figure S1; Jantzen, Kieffer-Olsen, & Madsen, 1994; Kieffer-Olsen, 1993). The commonly accepted date ranges for the four arm positions are A (1050–1250 AD), B (1250–1350 AD), C (1350–1450 AD), D (1450–1536 AD), with the latest position given temporal primacy in case of a mixed position (Boldsen, personal communication). When possible, dates have been refined by incorporating information from stratigraphy, cemetery duration, and other elements of burial such as grave type or cemetery area. Temporal designations from excavation reports were incorporated when

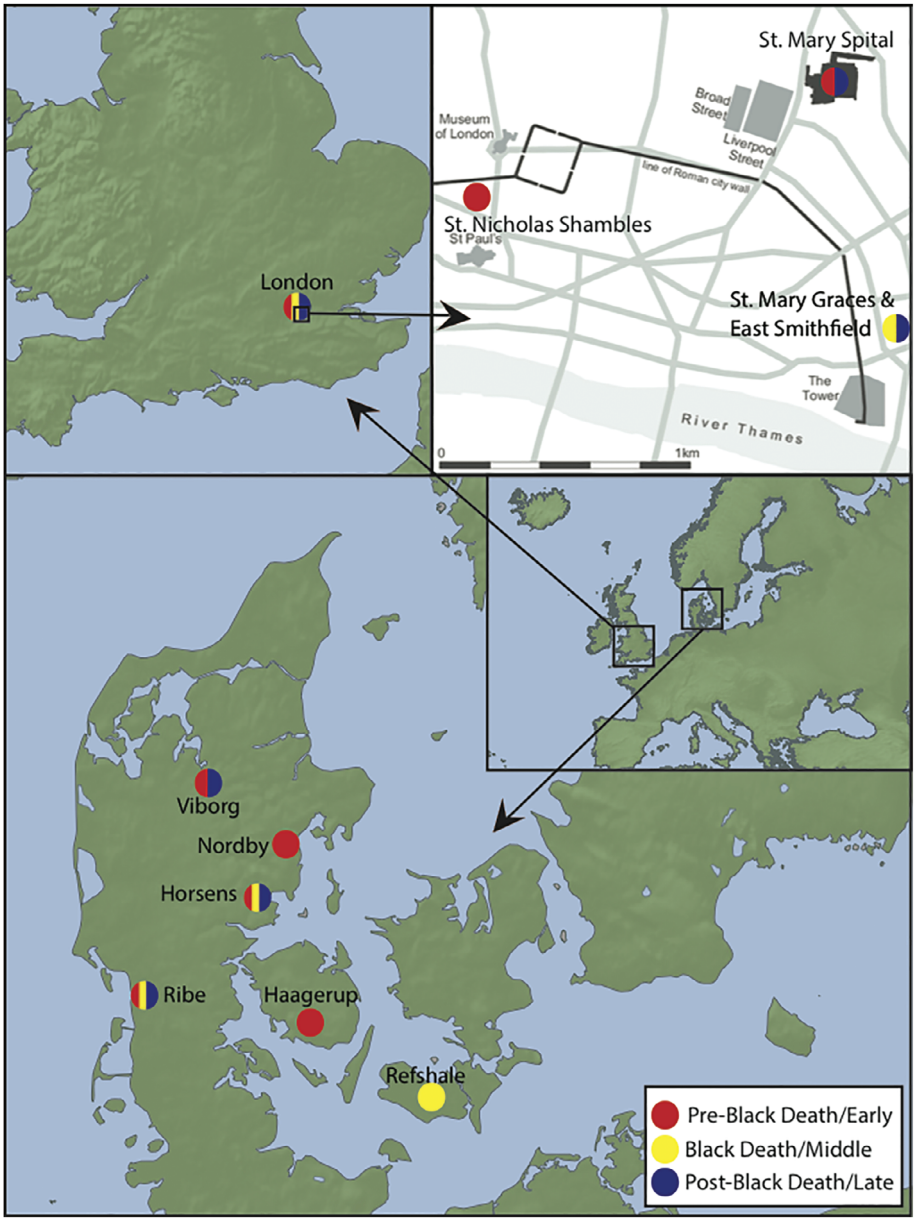

FIGURE 1 Danish and London cemetery locations from which individuals were obtained for this study. London maps after Sidell et al. 2007 © Museum of London Archeology

available. Unfortunately, the arm position technique does not always resolve dates sufficiently to securely classify individuals into before, during, or after Black Death groups, although it can be used to broadly relate time period of origin between individuals. The lack of Black Death mass burials in Denmark precludes secure identification of any individuals as coming from plague burials. Therefore, the Danish samples are classified into three time periods: early (before mid-14th century), middle (circa mid-14th century), and late (post-14th century). Samples that could not be categorized into a single period were not included in analyses that required temporal categorization, but are included when total Danish population diversity was assessed. Viking-era samples were classified as early and post-medieval samples were classified as late when Danish samples were broken into three temporal categories. When Danish and English samples are considered together as a late-medieval population, the time periods that broadly overlap are grouped: early and pre-Black Death are grouped, middle and Black Death are grouped, and late and post-Black Death are grouped. Individuals were sampled at random from both the English and Danish cemeteries.

2.2 | Sampling, DNA extraction, and targeted enrichment

We subsampled between 10 and 300 mg of the petrous portion of the temporal bone, tooth pulp cavity, or tooth root for DNA extractions (Figure 2). The subsample was crushed manually, demineralized, and digested according to previously published protocols (Schwarz et al., 2009). DNA was purified using a guanidinium hydrochloride-based buffer optimized to retain ultra-short fragments using a large-volume silica column (Roche; Dabney et al., 2013; Glocke & Meyer, 2017). Samples were assessed for inhibition and preservation of human nuclear DNA via a quantitative PCR (qPCR) assay designed to target the single copy nuclear cMYC gene based on Morin, Chambers, Boesch, and

TABLE 1 Sample summary, including age and geographic origin of individuals included in this study

Time period	Site code	Site name	Location	Urban or rural	Number of genomes
Early: Viking	ASR 2391/ASR 13 II	Ribe Lindegärden	Ribe, Denmark	Urban	4
Early	VSM 902F	Sct. Leonisgade/Sct. Drotten	Viborg, Denmark	Urban	1
	VSM 855F	Sct. Mathias	Viborg, Denmark	Urban	6
	ØHM 1247	Haagerup	Haagerup, Denmark	Rural	2
	VSM 09264	Skt. Drotten	Viborg, Denmark	Urban	1
	FHM 3970	Nordby	Nordby, Denmark	Rural	17
	ASR 1015	Ribe Gräbrødre	Ribe, Denmark	Urban	5
	HOM 1046	Sejet	Horsens, Denmark	Rural	3
Pre-Black Death	GPO75	St. Nicholas Shambles	London, UK	Urban	6
	SRP98, phase 15	St. Mary Spital	London, UK	Urban	33
Early/middle	VSM 29F	Faldborg	Viborg, Denmark	Rural	1
	HOM 1649	Ole Wormsgade	Horsens, Denmark	Urban	1
Middle	ASR 1015	Ribe Gräbrødre	Ribe, Denmark	Urban	1
	Refshale	Refshale	Refshale, Denmark	Rural	10
	HOM 1649	Ole Wormsgade	Horsens, Denmark	Urban	4
	VKH 1201	Tirup	Horsens, Denmark	Rural	7
	HOM 1046	Sejet	Horsens, Denmark	Rural	2
Black Death	MIN86, period 1	East Smithfield	London, UK	Urban	35
Middle/late	ASR 13 II	Ribe Lindegärden	Ribe, Denmark	Urban	7
	HOM 1046	Sejet	Horsens, Denmark	Rural	1
Late	VSM 902F	Sct. Leonisgade/Sct. Drotten	Viborg, Denmark	Urban	2
	ASR 1015	Ribe Gräbrødre	Ribe, Denmark	Urban	16
	VSM 29F	Faldborg	Viborg, Denmark	Rural	2
	ASR 13 II	Ribe Lindegärden	Ribe, Denmark	Urban	11
	HOM 1649	Ole Wormsgade	Horsens, Denmark	Urban	3
Post-Black Death	MIN86, period 2	St. Mary Graces	London, UK	Urban	28
	SRP98, phase 17	St. Mary Spital	London, UK	Urban	30
Late–postmedieval	HOM 1272	Klosterkirken	Horsens, Denmark	Urban	24
Early/middle/late	HOM 1649	Ole Wormsgade	Horsens, Denmark	Urban	1

Vigilant (2001), but with a different reverse primer to shorten the amplicon to 52 base pairs (bp) instead of the original 81 bp (See Supporting Information for primer sequences). Double-stranded, dual-indexed Illumina-style sequencing libraries were constructed from qPCR-positive extracts using previously published methods (Kircher, Sawyer, & Meyer, 2011; Meyer & Kircher, 2010).

Targeted capture was performed following the MYbaits v3 protocol (Arbor Biosciences, Ann Arbor, MI) with minor modifications to allow for an initial reaction volume of 26 μl, as detailed in the Supporting Information for a set of nuclear loci (Klunk, unpublished data). We saved the non-enriched portion of this nuclear enrichment, which is typically discarded, and subsequently used 25 μl as input to a new targeted capture reaction containing 1 μl of 50 ng/μl human mitochondrial baits without additional purification (Figure 2). The human mitochondrial baits were designed based on a global reference panel to minimize biases (designed by Ana Duggan and Hendrik Poinar based on database from Renaud, Slon, Duggan, & Kelso, 2015).

2.3 | Data analysis

All samples were sequenced to an average of 732,209 raw reads (range: 3,549–20,052,880) using 2 × 90 read chemistry on the Illumina HiSeq 1500 platform at the Farncombe Metagenomics Facility (McMaster University, Hamilton, ON, Canada). Adapters were trimmed and overlapping reads were merged using leeHom with aDNA specific settings (Renaud, Stenzel, & Kelso, 2014). Reads were mapped to the revised Cambridge Reference Sequence (rCRS) using a modified version of BWA (Li & Durbin, 2009; https://github.com/udo-stenzel/network-aware-bwa). PCR duplicates were removed with biohazard software (https://github.com/udo-stenzel/biohazard) and reads with mapping quality below 35 and length below 35 were removed with samtools.

Schmutzi was used to create consensus sequences and estimate levels of contamination (Renaud et al., 2015). If the consensus sequence generated by Schmutzi was composed of <10% Ns (missing data), it was considered to be complete and retained for further analysis. Haplotypes were called with Haplogrep 2.0, using Phylotree build 17 (van Oven, 2015; Weissensteiner et al., 2016). The consensus sequences were aligned with MAFFT (v7.205), poorly aligned regions were removed with trimAl (v1.4.rev15), and appropriate statistical models of evolution were chosen using ModelTest-NG (Capella-Gutiérrez, Silla-Martínez, & Gabaldón, 2009; Katoh & Standley, 2013; http://www.github.com/ddarriba/modeltest/). Arlequin (v 3.5) was used to perform population pairwise Φ_{ST} analysis (Excoffier & Lischer, 2010). Median-joining networks were constructed in Network (v5) with transversions:transitions weighted 3:1 and visualized in

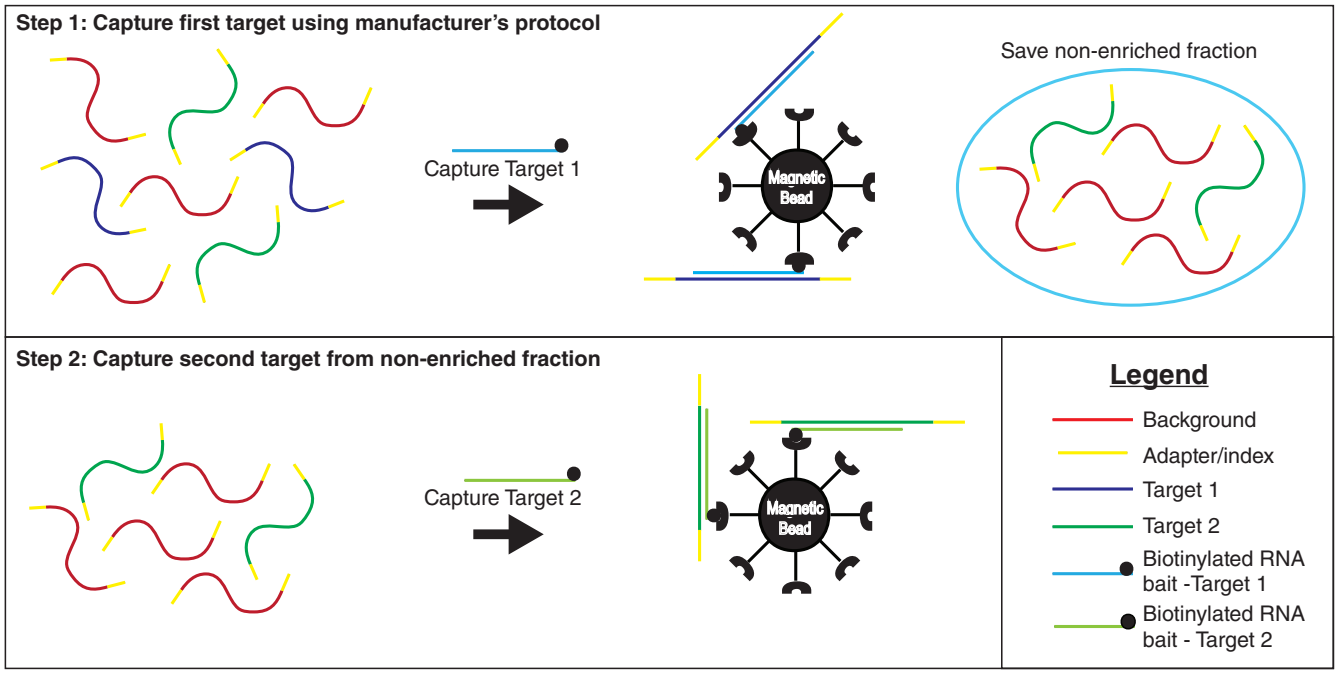

FIGURE 2 Simplified schematic of the novel strategy employed to capture mitochondrial DNA (target 2) from the nonenriched fraction of a different targeted capture experiment for nuclear DNA (target 1). Red fragments represent nontarget or background DNA, which makes up a large portion of ancient DNA extracts and can be captured through nonspecific hybridization

Network Publisher (http://www.fluxus-engineering.com/sharenet.htm). Maximum likelihood trees were constructed with RAxML (v8.0.25) using the GTRGAMMAIX model and visualized in FigTree (v1.4.0, http://tree.bio.ed.ac.uk/software/figtree/; Stamatakis, 2014). Damage pattern analysis was performed with mapDamage 2.0 and plotted in R using the ggplot2 package (Jónsson, Ginolhac, Schubert, Johnson, & Orlando, 2013; Wickham, 2016).

For comparative datasets, we used additional sequences from English populations that predate the late medieval period (dates ranging from 210 BC to 910 AD), early medieval Hungary (9th–11th centuries AD) as well as contemporary sequences from England, Denmark, Finland, Italy, Central Europeans from Utah, and the Caucasus (n = 802, Supporting Information Table S2). Comparative data was accessed either as publicly available consensus sequences or as mapped bam files, which were subsequently processed in the same manner as the data from this study to generate consensus sequences. Populations were required to have full mitochondrial genome data from at least 10 individuals. The modern Danish dataset was downsampled randomly from 2,000 individuals to 200 individuals (Li et al., 2014). The modern populations provide a European geographical comparison. The Finnish and Caucasian samples provide comparisons of populations that are expected to be genetically dissimilar. The Hungarian early medieval samples were the only population-level (early) medieval data available at the time of analysis. Finally, the British

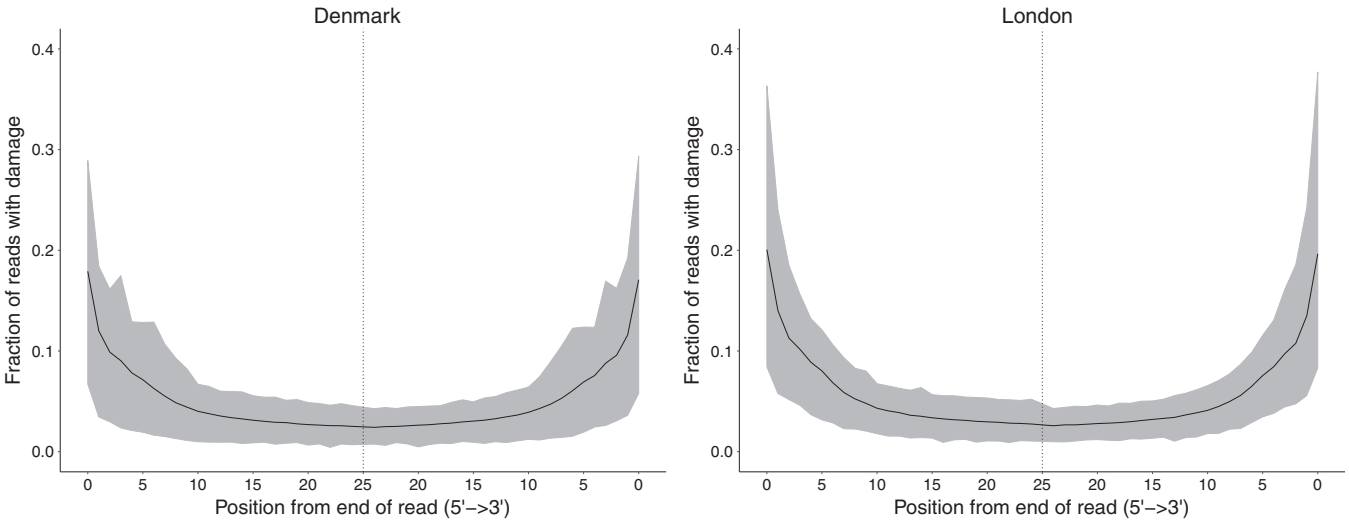

FIGURE 3 Damage pattern plot of all samples from London (left) and Denmark (right). The black line indicates the mean value and the gray shading indicates the minimum and maximum values at each position across all samples

samples predating those sequenced in this study are included to compare across the same geographic area over a longer time-scale. The alignments and analyses of the comparative sample set were performed as above. A multidimensional scaling plot was generated in R using the isoMDS function from the MASS package and plotted in R (Venables & Ripley, 2002). Bayesian Skyline analysis was performed using BEAST (version 1.7.5; Drummond, Suchard, Xie, & Rambaut, 2012).

3 | RESULTS

3.1 | Ancient DNA characterization

Using our novel capture strategy, we were able to reconstruct 264 complete mitochondrial genomes (132 from London and 132 from Denmark), from our starting set of 279 individuals, representing a success rate of 94.6%. Neither the reagent nor carrier blanks contained enough mappable reads to produce a consensus sequence (Supporting Information Table S1). Average coverage depth for our samples ranged from 19 to 2,456x with an average of 610x. All sequencing libraries display a damage pattern consistent with aDNA (Figure 3). Consensus sequences have been deposited to the NCBI GenBank archive (accessions MK059485–MK059748) and raw reads have been submitted to the NCBI Short Read Archive (BioProject PRJNA497483).

3.2 | Mitochondrial DNA haplogroup assignment and haplotypic diversity

The haplogroups present in both medieval sample sets are common amongst contemporary Europeans, with the exception of a single middle/late period Danish individual with haplogroup C4a1a and a single Viking-era Danish individual with haplogroup N1b1a (Figure 4, Supporting Information Figures S2 and S3, Supporting Information - Table S3 and S4). Haplogroup C4a1a has been found in a modern sample from Poland but has a higher frequency in northern, central, and eastern Asia (Mielnik-Sikorska et al., 2013). Haplogroup N1b1a has previously been found in an individual from Ukraine, dating to the 7th century BC and may be associated with the Neolithic expansion (Juras et al., 2017). There are very few exact sequences shared between samples; there is only one exact sequence match between an individual from pre-Black Death London and an individual from late period Denmark. This indicates a lack of individuals who are close maternal relations in our sample set and agrees with historical interpretations of large population size. This lack of exact sequence sharing can be visualized through median-joining networks (Supporting Information Figures S4–S10). However, there are several pairs of individuals that share haplotype assignments, despite not having identical sequences. Without dividing populations based on the time periods assigned above, there are 14 haplotypes shared between 31 individuals in London, 18 haplotypes shared between 37 individuals in Denmark, and 21 haplotypes shared between 58 individuals across both locations. This final set includes those that were shared within a single population, but are also shared with one or more individuals from the other population (Supporting Information Figure S11).

In order to explore whether missing data (Ns) were driving the high number of unique sequences, we removed all positions containing an N in one or more sequences and then compared exact sequence matches within and between populations. Before accounting for missing data, there were no identical sequences within the London or the Danish populations, and only one match between both. After removing all positions containing an N in one or more of the sequences we were left with a total of 14,288 bp for the London dataset and 16,055 bp for the Danish dataset and 14,264 bp in the combined dataset. After this cleaning, there remained three pairs of identical sequences within the London dataset, five pairs of identical sequences within the Danish dataset, and six identical sequence pairs and one identical sequence trio (which included a Danish pair) within the combined datasets (Supporting Information Table S22). This analysis suggests that the missing data are minimally contributing to, but clearly not driving the observed sequence diversity.

3.3 | Comparing genetic diversity through population pairwise Φ_{ST} analysis

To examine differences in genetic diversity between datasets, we calculated population pairwise Φ_{ST} values using a series of groupings in Arlequin (Table 2). First, we compared individuals within each location over time and found no statistically significant differences. In addition, Φ_{ST} values were negative or extremely close to zero, suggesting very similar genetic composition within each geographical location (Supporting Information Tables S5–S7). We then performed the same analyses alternately grouping the Black Death (London) or middle period (Denmark) individuals with either the pre-Black Death/early period or post-Black Death/late period, resulting in similar nonsignificant values (Supporting Information Tables S8–S11). Next, to determine whether there was a signal of increased female migration after the Black Death that may have been masked by the inclusion of males in the dataset, we divided the London population into males and females based on the morphological sexing score, and repeated the Φ_{ST} analysis (Supporting Information Tables S12 and S13). We performed the analysis twice: once for individuals that had been previously classified with high likelihood as males and females (morphological scores of 1 and 5, respectively), and then again including individuals previously classified as likely males and females (morphological scores of 2 and 4, respectively; WORD Database, 2017; Center for Human Bioarchaeology, 2013a, 2013b, SRP98 workbooks). Individuals who were subadults, of indeterminate sex or unclassified could not be included in this analysis. Despite this reduction in the size of the dataset, there remained no statistically significant difference in the genetic diversity of the sample sets. We then compared rural and urban populations in Denmark, without dividing individuals based on time, to test whether or not the populations were genetically distinguishable, and again, the results indicated no significant difference (Supporting Information Table S14). Dividing the rural and urban individuals into time periods also produced no significant differences (Supporting Information Table S15). While the population sizes for the rural late period (n = 3) and urban middle period (n = 5) groupings are too small for meaningful comparisons, all other remaining groups (4) were large enough to be meaningful.

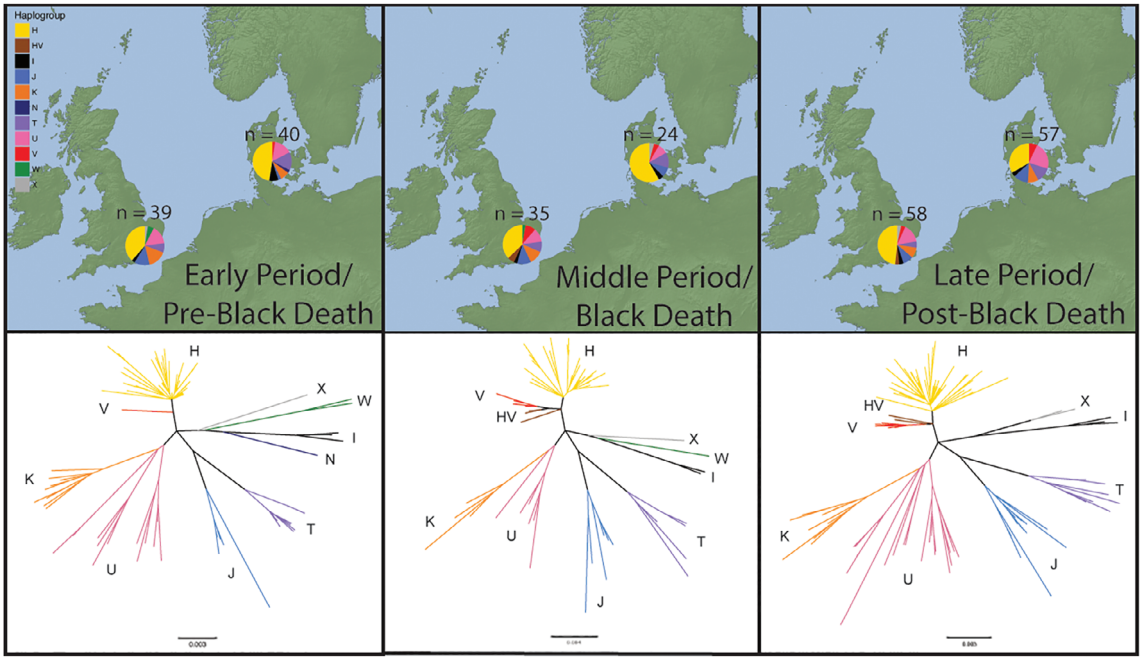

FIGURE 4 Distribution of haplogroups through the late medieval period in London and Denmark (top row). Maximum likelihood trees of haplotypic diversity in both sample sets across time (bottom row). Danish individuals that cannot be assigned to a single period are not included in this figure (n=11)

We also performed Φ_{ST} analysis comparing the individuals from London to those from Denmark by splitting them into three or five time-based groups and still found no significant differences (Supporting Information Tables S16 and S17). Next, we treated the medieval individuals as a single population, split into three time periods, and found no significant differences between groups (Supporting Information Table S18). Finally, we compared our sequences, divided into three-time categories (before, during, and after the Black Death for London and early, middle, late for Denmark), with our larger comparative dataset of samples from contemporary and ancient Europe. The only significant differences were between the Western European groups and modern Finland and the Caucasus, as well as early medieval Hungary (Supporting Information Table S19). We visualized these results through a multidimensional scaling plot, which highlights the high degree of similarity of the Western European groups, regardless of time (Figure 5). ModelTest-NG results for each group of sequences can be found in Supporting Information Table S20.

TABLE 2 Summary of population pairwise Φ_{ST} tests and results

Sequences used	Grouping	*p* value <0.05
London	Three-time points	Not detected
London	Males and females, three-time points	Not detected
Denmark	Three-time points	Not detected
Denmark	Five-time points	Not detected
Denmark	Urban and rural	Not detected
London + Denmark	Three-time points	Not detected
London + Denmark	London three time points, Denmark five time points	Not detected
London + Denmark + Supporting Information Table S2 sequences	Three-time points	Medieval Hungary, Modern Finland, Modern Caucasus[a]

[a]Not all time-separated groups were significantly different; see Supporting Information Table S19.

3.4 | Estimating demographic history with Bayesian skyline analysis

We generated a Bayesian skyline plot in order to examine the demographic history of our samples. In the resulting plots, we see no population size contraction over the period including the Black Death (Supporting Information Figure S12). This could indicate that the Black Death had a smaller mortality rate than previously estimated based on historical studies. However, we believe this result is more likely driven by our sample size, which is relatively small compared to the population sizes of medieval Europe. As we have sampled only a small fraction of the total diversity from the large effective population size of mitochondrial genomes in medieval Europe, we believe this analysis results in essentially a reconstruction of the population history for common European mitochondrial haplogroups.

4 | DISCUSSION

4.1 | Success of non-enriched fraction capture strategy

We employed a successful and novel capture strategy to recover complete mitochondrial genomes from the nonenriched fraction of an initial capture reaction, which is typically discarded as waste. This technique saves sample volume, as it does not require fresh library volume for the second enrichment. It also conserves reagents, as new buffers are not required before performing the enrichment for the second target. In order to assess complexity, the unmapped, merged

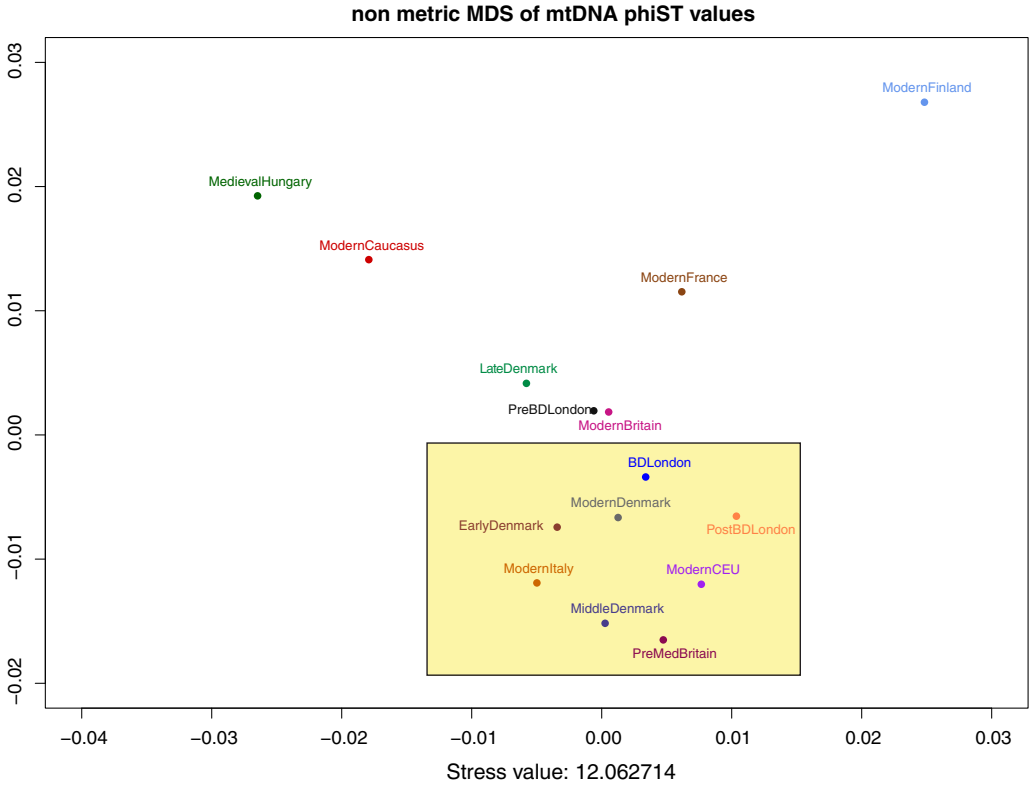

FIGURE 5 Multidimensional scaling plot of all samples used for comparative analysis with Danish and London samples split into the respective three time periods. All pairs within the box are artificially separated for clarity due to negative or zero Φ_{ST} values. Several other individual pairs are also artificially separated for the same reasons. Artificial separation entailed correcting negative Φ_{ST} values to zero and subsequently adding 0.0001 to zero values. Refer to Supporting Information Table S2 for more information

reads were sampled to a depth of 10,000 reads, and then the proportion of reads of at least 35 bp in length and mapping quality of 35 that were uniquely mapped to the rCRS was assessed. This depth was chosen because it resulted in an average coverage depth of 29.7x (range: 5.13–45.3x), which would be able to produce consensus sequences using our quality filtering for the majority of samples. The average unique percentage of mapped reads is 68.51% and the range is 13.20–89.47%, which represents a very high complexity. Because of these results, we recommend saving the nonenriched fraction in order to minimize the destructive sampling to a single sample while maximizing the information that can be gleaned from each individual.

4.2 | Mitogenomic continuity in medieval London and Denmark

We found no evidence for mitogenomic discontinuity after the Black Death in populations from London or Denmark. Rather than a lack of migration in this period, these data suggest that the diversity was likely very high, preventing clear detection of signatures of migration using mitochondrial genomes alone. It is clear that if a large migration from outlying rural areas into urban centers had taken place in the later medieval period, the migrant or source population was not genetically distinct from the urban population(s) they were replacing. The lack of genetic differences between Danish rural and urban cemeteries supports this theory. Another finding that supports this hypothesis is the mitogenomic similarity of all geographically disparate British samples included in the analysis, which span a time frame of approximately 2,200 years. This suggests that the mitochondrial genetic diversity within Britain has not changed significantly during this period of time. It is worth noting that the modern British samples come from individuals from whom all four grandparents came from rural areas within 40 miles of one another, so the full modern diversity of the British population is likely not represented in this dataset. The lack of genetic dissimilarity in samples from wider geographic areas within Europe suggests that migrants from even further locales may not be detectable with mitochondrial data. This point is illustrated in Figure 5, a multidimensional scaling plot, as many of the European sample groups are so genetically similar, they would be plotted directly on top of each other, and therefore required an artificial separation for visualization. From a mitochondrial standpoint, Europe, here defined by our limited sample set, appears to have been relatively homogenous in terms of diversity throughout the medieval period.

4.3 | Migration and population replacement after the Black Death

After the major population losses of the 14th century, the total population numbers did not come close to reaching pre-Black Death numbers for several centuries, in part due to subsequent epidemics during the second plague pandemic (1346–1722 AD; Dyer, 2000; Hybel & Poulsen, 2007). Interestingly, while the population of Denmark as a whole decreased and growth stagnated after the Black Death, with overall population numbers not reaching pre-Black Death levels until the 19th century AD, urban centers rebounded much more quickly

and actually started to grow by the beginning of the 15th century AD (Hybel & Poulsen, 2007). The (predominantly slow) population growth in London and Denmark after the Black Death can be explained in three ways: (a) replacement by offspring of local survivors, (b) migration from outside sources, or (c) some combination thereof. Population growth via replacement with offspring of local survivors postpandemic should result in an increase in haplotype sharing after the Black Death, as more people would be expected to be maternally related due to fewer individuals contributing a greater proportion to the next generation. The trend in both London and Denmark is an increase of haplotype sharing in the post-Black Death/late period group relative to the pre-Black Death/early period group, but it is not statistically significant (Supporting Information Table S21). The overall increase in haplotype sharing does suggest that local survivors of the pandemic were indeed contributing to population replacement.

On the other hand, if migrants from a distinct population with little prior interbreeding were contributing to population growth, we would expect to see an influx of novel haplotypes and potentially a change in overall genetic diversity. The number of novel haplotypes present in the population does not increase over time in either Denmark or London (Fisher's exact test for count data p value is greater than 0.75 in both cases). Because the genetic diversity does not significantly change and the number of novel haplotypes does not significantly increase, it is unlikely that the population grew due to receiving a large number of migrants from an unrelated population. This interpretation is also supported historically, as the majority of apprentices in London during the 14th century AD came from the home counties (i.e., the counties surrounding the city) and in the 15th century the range expanded to additional northern and western counties, which still most likely represent related populations (Hanawalt, 1993). Finally, if migrants were traveling from a source population that was closely related to the endpoint population, we would expect to see some novel haplotypes, but also some shared haplotypes, and perhaps would not see a change in genetic diversity. A contribution from local survivors would not be distinguishable in this scenario. This interpretation most closely matches these data.

Exact sequence matches can indicate close maternal lineage relationships, but the precise nature of these relationships cannot be determined. When we remove positions with missing data, we find 13 overall sets of exact sequence matches, one of which involves three individuals (Supporting Information Table S22). What is striking about the matches is that only two of the 13 matches involve individuals buried in the same cemetery in the same time period—one pair from Refshale in Denmark during the middle period and one pair from St. Mary Graces in London after the Black Death. It is important to note, however, that both of these cemeteries were in use for over a century, so the burials may or may not be contemporaneous. However, it is interesting that the Danish cemeteries that served rural communities, such as Tirup, which would have been composed of, on average, approximately 75 individuals at any one time (Boldsen, 2000), had no individuals with identical sequences. These rural cemeteries would have most likely included burials of familial groups, so it is potentially surprising that our sampling has picked up few closely maternally related individuals. For the cemeteries that did not serve specific parishes in London, such as St. Mary Spital (which served pilgrims, the infirm, and the pregnant women as well as monks, lay nuns, and wealthy benefactors) or East Smithfield (for which the catchment area is unknown), it is not surprising that there are few individuals who are close maternal relations (Connell et al., 2012; Grainger et al., 2008). Of the 13 total exact sequence-matching pairs, 4 are exclusively between Danish samples, 3 are exclusively between London samples, and 6 are between Danish and London samples. The relatively large number of related individuals between Denmark and London may be evidence of the shared history between England and Denmark, namely through Viking invasions of England starting in the late 8th century AD and culminating in the rule of England, Denmark, and Norway by a single king, Cnut, in the early 11th century AD (Richards, 2004; Williams, 1986).

4.4 | Integrating DNA sequence data with isotopic and documentary evidence

While we cannot determine the mechanism of population growth after the Black Death solely with mtDNA, when integrating our data with other types of evidence we can nonetheless begin to paint a fuller picture of demography in this tumultuous time period. Stable isotope studies have identified potential migrants in London and two cemeteries in Denmark, but have placed the majority of migrants' origins relatively close, geographically, to their place of burial, although some individuals in the East Smithfield dataset were found to have slightly more distant origins in western Britain (Duignan, 2015; Gough, 2014; Kendall et al., 2013). Documentary studies provide evidence that people were moving to urban centers such as London from not only proximate but also from distant locations (Goldberg, 2004). For example, there are 17,376 recorded instances of “resident aliens” in London between 1336 and 1584 AD (Lutkin, 2016). Taken together, the data from these studies and ours suggest that the population grew in the centuries post-Black Death due to a combination of replacement from both local sources and migrants stemming from rural sources and sources further afield. They do not support long distance immigration.

Given the homogeneity we observe in haplogroup composition between our medieval populations and contemporary Europeans, it would be difficult to identify a European migrant based on mtDNA alone. For example, when we look at 14 populations from across Europe today, we find no significant difference in the distribution of the major European haplogroups using Fisher's exact test for count data (Supporting Information Table S4). When we add our medieval samples to this pool, there continues to be no significant difference in the distribution of haplogroups (Supporting Information Table S4). Testing the hypothesis that the post-Black Death urban population was composed of both descendants of local Black Death survivors and migrants from varying distances would require sequencing more complete mitochondrial genomes from individuals from additional locations in medieval Europe, nuclear DNA from a large number of medieval individuals, and/or additional isotopic (i.e., strontium, oxygen, lead) data.

4.5 | Female migration in medieval northwestern Europe

Multiple additional lines of evidence suggest that females were migrating more frequently than males after the Black Death in order

to take advantage of the new economic opportunities in larger urban centers. This evidence comes from several sources, for example, the disparity in diet observed between young and older females in London (Lakin, 2010; Walter, 2017). Additional sources show an elevated risk in mortality in urban contexts as compared to the rural context for females in late medieval London (Walter & DeWitte, 2017) and in medieval Viborg (Boldsen, 1984). Finally, evidence for post-Black Death female migration comes from changing demographic profiles between urban and rural contexts in England showing an increase in younger females in urban environments after the Black Death (Lewis, 2016). Working as a servant or wage laborer during adolescence became a common stage in the life cycle of women after the Black Death due to rising wages and a shift away from agricultural cultivation (Goldberg, 1992). Evidence from the poll tax of 1377 AD in England reveals there were a greater number of female servants than male servants in towns (Kowaleski, 2014). The increased prevalence of female wage earners in urban centers may have also influenced marriage patterns in northwestern Europe toward an overall decrease in marriage frequency, a more autonomous choice for women, and a higher average age, instigating a new pattern of neolocality as opposed to patrilocality (De Moor & Van Zanden, 2010; Kowaleski, 2013). It was not unusual for women to return to their rural home village to marry after working a few years as a wage laborer, suggesting a greater complexity of female migration patterns than a simple rural-to-urban model (Bitel, 2002; Goldberg, 2004).

Because mtDNA is only maternally inherited, our data can address female population diversity (and matrilocality or lack thereof) in a way that other types of DNA evidence cannot. If the frequency of female movement between rural and urban environments increased after the Black Death, and the populations had different or divergent haplotype frequencies, we would expect to see differences in genetic diversity over time as more migrants made their way into urban environments. However, we do not observe changes in genetic diversity over time, neither in the single urban context of London nor in the wider geographic context of Denmark. One interpretation of this lack of genetic difference is that females were migrating at high frequencies throughout the medieval period, even prior to the Black Death. One historical study looking at court records in the Midland's town of Halesowen (England) from 1272 to 1350 AD found that three-quarters of the migrants into the town were women, although the validity of these data has been questioned (Goldberg, 1992; Hilton, 1982). While this evidence is uncertain, the dramatic differences illustrate the disparity of migration patterns between the sexes, even if some overestimation occurred. Migration from rural to urban environments before the Black Death was definitely occurring, especially for the poor, but it was the scarcity of economic opportunities in non-urban areas that was the major driver of movement, instead of increased opportunities (Goldberg, 1992; Kowaleski, 2013). One potential driver of female migration in Denmark in the medieval period was the increased strictness of religious laws surrounding consanguinity and marriage beginning in the early medieval period (Cirivilleri, 2000). While there are no written records on potential systems of bridal exchange in rural Denmark, the necessity for females to marry outside of a closely related small village that was stipulated by these regulations may have influenced genetic diversity by increasing effective population size in relation to the actual population size (Boldsen, 1989). Our mitochondrial genetic evidence supports female migration before and after the Black Death in that there is a great deal of within-population haplotypic diversity and the overall diversity does not change through time. However, the combination of the high degree of genetic diversity present in each time period and the lack of temporal changes in this diversity may also be an artifact of a large initial population size, of which we are only sampling a small fraction.

In this study, we provide the first glimpse of population-level mitogenomic diversity in London and Denmark during the late medieval period from 264 individuals. We captured these data using a novel strategy that targets the nonenriched fraction of a previous enrichment, which is typically discarded as waste, thereby conserving precious library and reducing cost. We find evidence for maternal genetic continuity before, during, and after the Black Death in London and throughout the medieval period in Denmark. We were unable to find direct evidence of large-scale population turnover using the mtDNA alone, but conclude that our data support a high frequency of female migration throughout the medieval period. Our data, in conjunction with those previously obtained from contemporary European populations, suggest that Europe maintained a diverse and relatively homogenous mitochondrial population throughout the last 1,000 years.

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ORCID

Jennifer Klunk https://orcid.org/0000-0002-6521-8516
Hendrik N. Poinar https://orcid.org/0000-0002-0314-4160

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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